IN VITRO STUDIES OF DIFFERENT TYPES OF CARBON NANOTUBES DEPOSITED ON PTFE MEMBRANE

Aneta Fraczek-Szczypta[1]*, Elzbieta Menaszek[1,2], Magdalena Miranowicz[1], Stanislaw Blazewicz[1]

[1]AGH-University of Science and Technology,
Faculty of Materials Science and Ceramics,
Department of Biomaterials,
30 Mickiewicza Ave, 30-059 Krakow, Poland
[2] Jagiellonian University-Collegium Medicum,
Department of Cytobiology,
9 Medyczna Str, 30-068 Krakow, Poland
*MAILTO: afraczek@agh.edu.pl

[Engineering of Biomaterials, 116-117,(2012), 153-154]

Introduction

Carbon nanotubes (CNTs) are considered as a promising material for medical applications. In biological systems, they have been investigated as drug delivery vehicles, targeted cancer therapies, tissue scaffolds, and biosensors [1–3]. CNTs due to their relative large length-to-diameter aspect ratio, with a very large specific surfaceare suitable for highly sensitive molecular detection andrecognition.Analysis of the available literature shows also positive influence of CNTs on cells proliferation and adhesion. Especially, bone and nerve cells are stimulated to growth in direct contact with CNTs or with materials such as polymer and ceramic which are modified with CNTs [4-6].

The aim of this study is to compare theeffects of differ-enttypes ofnanotubesdeposited on polytetrafluoroethylene (PTFE) membrane oncellularresponse. Four types of as-prepared CNTs differ in structure, diameter, length and purity were investigated in contact with the human osteoblast (NHOst). Additionally, the influence of CNTs on cells viability and their morphological condition in comparison with pure PTFE membrane was investigated.

Materials and methods

Four types of as-prepared CNTswere investigated in this work. Three types of them were provided by NanoAmorUSA and NanoCraftInc USA and one was synthesis using chemical vapour deposition (CVD) method at the Department of Biomaterials (AGH, Krakow). Carbon nanotubes were synthesis in quartz tube furnace where as a substrate for CNTs growth, the silicon wafer was used. This substrate was covered by nickel (Ni) which was used as acatalyst forthe growthof nanotubes. The carbon source and carrier gases were acetylene (C_2H_2) and nitrogen (N_2), respectively. CNTs were synthesized at 750^{o}C. In this temperature the mixture of C_2H_2 and N_2 was injected for 25min with a flow 2l/h and 15l/h, respectively. All types of CNTs are shown in TEM microphotographs (FIG.1).The pristine CNTs used in this experiment were denoted, as follows:

- MWCNT-A – multi wall carbon nanotubes obtained at AGH;
- MWCNT – multi wall carbon nanotubes obtained from NanoAmorUSA;
- SWCNH – single wall carbon nanohorns obtained from NanoCraftInc USA;
- ULSWCNT – ultra long single wall carbon nanotubes obtained from NanoAmorUSA.

For biocompatibility investigation, the CNTs were deposited on PTFE membrane filter (Whatman PTFE membrane) and then cultured with human osteoblast cells (NHOst) (Lonza, # CC-2538) for 3 and 7 days. To determine cytotoxicity of CNTs in contact with osteoblast ToxiLight®BioAssay Kit tests (LONZA Rockland, Inc.) were used. The interaction of nanotubes with NHOst was observed using a scanning electron microscopy (SEM, Nova NanoSEM 200, FEI).

The morphology of CNTs was analysed using transmission electron microscopy (TEM) (Tecnai G2 F20 (200 kV) and Joel). The degree of purification of CNTs was determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (Multiwave 3000, Perkin Elmer Co.) and energy dispersive spectrometry (EDS) (EDAX Co.). The contact angle was measurement by sessile drop method by an automatic drop shape analysis system DSA 10 Mk2 (Kruss, Germany).

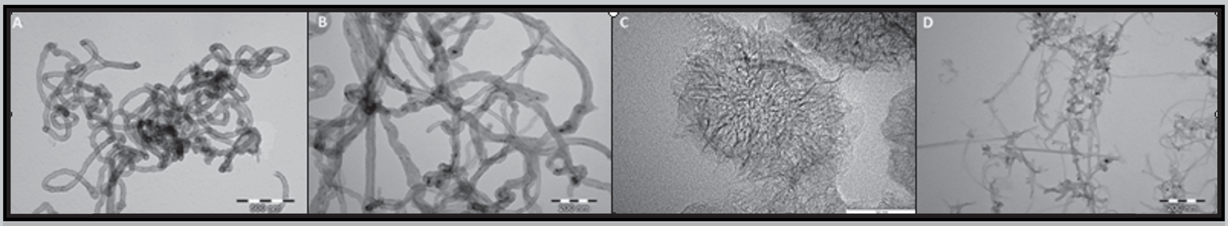

FIG.1. TEM microphotographs of MWCNT-A (A), MWCNT (B), SWCNH (C) and ULSWCNT (D)

Results and discussion

Carbon nanotubes used in this work possess different dimension and purity (TABLE 1). ICP-OES and EDS analysis of the as-prepared MWCNTand MWCNT-A indicated mainly the presence of nickel (Ni) catalyst in this sample. In the case of the pristine SWCNH and ULSWCNT, the ICP-OES analysis showed mainly the presence of iron (Fe) and cobalt (Co), respectively. Wettability of CNTs deposited on PTFE membrane is higher in comparison with polymer membrane (TABLE 1). Hydrophobic nature of CNTs and their ability to nanostructure the surface causes that the substrates modified by CNTs may possess the super hydrophobic nature. Super hydrophobic properties were especially observed for samples modified with MWCNT-As and ULSWCNTs.

Biological results show that membranes covered with CNTs have lower cytotoxicity in comparison with pure

TABLE 1. Parameters characterizing CNTs.

	Diameter [nm]	Length [μm]	Purity [%]	Wettability [°]
MWCNT-A	50-70	2-5	0,9 (Ni) (EDS)	156,6±8,7
MWCNT	10–30	1-2	1,2 (Ni) (ICP)	146,7±6,6
SWCNH	2-3	30-50	1,8 (Fe) (ICP)	148,0±5,5
ULSWCNT	0,7-2	15-30	0,8 (Co) (ICP)	154,4±5,6
PTFE membrane	-	-	-	140,6±3,8

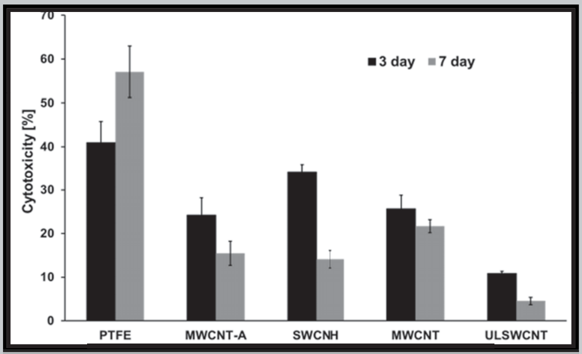

FIG. 2. Cytotoxicity of osteoblast in contact with pure PTFE membrane and modified with CNTs after three and seven days of incubation.

PTFE membrane what indicated that presence of this nanomaterialsimprove biocompatibility of polymer membrane (FIG.2). The lowest cytotoxicity was observed for samples containing ULSWCNTs and MWCNT-As;for thosefor which thecontact anglewas the highest.An interesting resultis also that cytotoxicity for membranes with CNTs decrease with time of cells culture, whereas for pure PTFE increase. The cells morphology in contact with samples is the confirmation of quantitative analysis (FIG.3). Not only cells morphology which is typical for normal osteoblastsbut also good adhesion and spreading indicates that CNTs improve biocompatibility of PTFE membrane in contact with bone cells. For comparison,the cells morphologyon the PTFEis shownin FIG.3A.

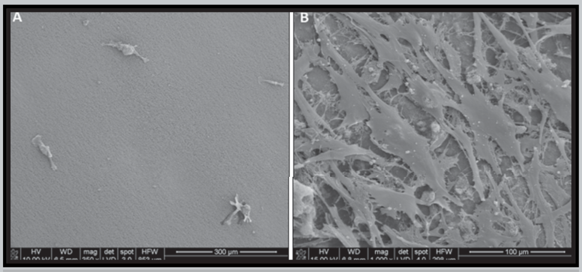

FIG. 3. SEM microphotographs of cells in contact with PTFE membrane (A) and PTFE membrane modified with MWCNTs (B).

The presence of metal catalysts in CNTs mayindirectlyaffect thecellular response. Especially Fe and Ni are known toadversely affect thecellular response. Analysing the samples with CNTs the highest cytotoxicity is observed for SWCNHs, MWCNTs and MWCNT-As where the iron and nickel are main contamination. The lowest cytotoxicity have sample where cobalt was used as catalyst (ULSWCNTs).

Conclusions

Four kinds of as-prepared CNTs deposited on PTFE membranes were contacted with human osteoblast to verify their cytotoxicity. All kinds of CNTs have positive impact on cells growth and proliferation and simultaneously reduce cytotoxicity in comparison with pure PTFE sample. An interestingresult isthat the highlyhydrophobicsurface of samples modified with CNTs has a positive effect on osteoblast morphology and adhesion. Probably not only wettability has impact on cell growth but also another factors such as nanostructuring. Resultsalso showthat the type ofcatalyst usedduring the synthesis ofCNTscanhave an impacton cellular response.The results arepromising, but need further analysisand verificationof other factorsthat may affect thecellular response.

Acknowledgements

This study has been supported by the Polish Ministry of Science and Higher Education, project no NN 507402039

References

[1] A. M. Schrand, L. Dai, J. J. Schlager, S. M. Hussain, and E. Osawa: Differential biocompatibility of carbon nanotubes and nanodiamonds.Diamond and Related Materials16 (2007) 2118-2123.
[2] J. Xu, M. Yudasaka, S. Kouraba, M. Sekido, Y. Yamamoto, and S. Iijima: Single wall carbon nanohorn as a drug carrier for controlled release.Chemical Physics Letters 461 (2008) 189-192.
[3] B. S. Harrison and A. Atala: Carbon nanotube applications for tissue engineering.Biomaterials28 (2007) 344-353.
[4] J. Beuvelot, C. Bergeret, R. Mallet, V. Fernandez, J. Cousseau, M. F. Baslé, D.Chappard:In vitro calcification of chemically functionalized carbon nanotubes. ActaBiomaterialia 6 (2010) 4110-4117.
[5]K. Sahithi, M. Swetha, K. Ramasamy, N. Srinivasan, N. Selvamurugan:Polymeric composites containing carbon nanotubes for bone tissue engineering. International Journal of Biological Macromolecules 46 (2010) 281-283.
[6]F. Tavangarian, Y. Li: Carbon nanostructures as nerve scaffolds for repairing large gaps in severed nerves. Ceramics International 38 (2012) 6075-6090.